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Contrasting Patterns of Phenotype-Dependent Parasitism within and among Populations of Threespine Stickleback

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ABSTRACT: Variation in infection rate arises from variation in host exposure and resistance to parasites both within and among populations. All things being equal, phenotypes that increase exposure risk should covary positively with infection among individuals. It might therefore be expected that populations with mean phenotypes that increase exposure might also have higher rates of infection. However, such positive covariance between exposure and infection at the population level might be undermined by other factors such as geographic variation in parasite abundance or host resistance, negating or reversing in between-population comparisons. We studied rates of infection of two parasites among 18 populations of threespine stickleback (*Gasterosteus aculeatus*). As predicted, within populations, trophic morphology covaries with infection of two trophically transmitted parasites: individuals with benthic (or limnetic) phenotypes were more likely to be infected with a benthic (or limnetic) parasite. However, across populations, the relationship between morphology and infection rate was absent (limnetic parasite) or reversed (benthic parasite). Our results confirm the importance of phenotype-dependent exposure, but stress different factors or processes, such as the evolution of reduced susceptibility, might shape variation in infection at larger spatial scales.

Keywords: parasite exposure, *Gasterosteus aculeatus*, generalized linear mixed model (GLMM), resistance, *Schistocephalus solidus*, spatial scale.

Introduction

Both within and among populations, hosts differ in their propensities to become infected with parasites (Schmid-Hempel 2011). One way such differences arise is through among-host variation in susceptibility to infection, a composite trait combining all the factors determining a host's likelihood of infection. For example, both genetic resistance and environmental variation in host condition or immunological state can cause differences in susceptibility among hosts (Lazzaro and Little 2009). Hosts may also differ in their tolerance of infection, with more tolerant hosts harboring relatively more parasites (or surviving longer) than less tolerant ones, despite equal susceptibility (Jokela et al. 2000; Roy and Kirchner 2000). Hosts can also vary substantially in their exposure to parasites in the environment (Keymer and Anderson 1979; Karvonen et al. 2004; Hechinger and Lafferty 2005; Poulin 2006). Exposure variation is a potentially important driver of infection differences because it acts before other mechanisms in determining hosts' likelihood of infection (Holmes 1987; Combes 2001). Thus, explaining how exposure rates differ within and among populations can potentially affect our understanding of many ecological and evolutionary phenomena involving parasitic infection (Anderson and May 1978; Woolhouse et al. 1997; Schmid-Hempel and Ebert 2003; Thompson 2005; Koskella and Lively 2007; Wood et al. 2007; Lafferty et al. 2008).

Infection is often positively correlated with features of the environment that increase exposure for all individuals within a single population. For parasites with complex (multihost) life histories, variation in exposure is often determined by the abundance of intermediate or terminal hosts. For example, highly acidic lakes serve as poor habitats for gastropods, which are the first hosts for many parasites of fish, and thus fish parasite communities can differ substantially across pH gradients (Curtis and Rau 1980; Marcogliese and Cone 1996). Populations can also differ in parasite exposure if their respective communities differ in the abundance of definitive host species that spread infective stages (Hechinger and Lafferty 2005; Byers et al. 2008) or of suitable alternate hosts that serve as transmission agents (Perkins et al. 2006; Johnson and Thieltges 2010; Ostfeld and Keesing 2012). Abiotic environmental variation can also directly affect the viability of free-living parasite stages, altering rates of exposure for their hosts (Pietrock and Marcogliese 2003).

Although exposure variation among individuals within

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a single population or geographic location is often stochastic, individual host phenotypes can also influence exposure rate in a number of ways (Lozano 1991; Barber et al. 2000; Hudson et al. 2002; Poulin 2006). Most directly, variation in avoidance behavior among individuals may generate variation in exposure, such as when exposure is tied directly to feeding behavior (Lozano 1991). Parker et al (2010) showed that gypsy moth (*Lymantria dispar*) larvae exhibit heritable variation in their propensity to consume foliage contaminated by conspecific cadavers infected with baculovirus. Host organisms may also be able to avoid microhabitats where exposure is more likely (Barber et al. 2000), as when stickleback fish (*Gasterosteus aculeatus* and *Gasterosteus wheatlandii*) adjust their microhabitat preference to avoid ectoparasites (Poulin and FitzGerald 1989). Organisms may also be able to avoid infected conspecifics as a way of reducing exposure (Barber et al. 2000, p. 200), for example, by reducing shoaling or grouping behavior, or avoiding infected potential mates (Hillgarth 1996).

Variation in parasite exposure can also occur as an incidental by-product of phenotypic variation resulting from other ecological interactions and processes. For example, variation in prey choice can lead directly to variation in exposure to parasites transmitted through particular prey or food items (Lozano 1991; Bolnick et al. 2003; Hall et al. 2007; Knudsen et al. 2011; Luong et al. 2013). Diet-related variation in parasitism has been most clearly noted in populations with discrete trophic polymorphisms (typically fish in north temperate or subarctic lakes) and/or in incipient species pairs in *Salvelinus* spp. (Walker et al. 1988; Frandsen et al. 1989; Dorucu et al. 1995; Knudsen et al. 1997, 2004, 2010, 2011; Bertrand et al. 2008), *Coregonus* spp. (Knudsen et al. 2003; Karvonen et al. 2013), *Lepomis macrochirus* (Wilson et al. 1996), Lake Malawi and Lake Victoria cichlids (Blais et al. 2007; Maan et al. 2008), and *G. aculeatus* (MacColl 2009). In these cases, two or more distinct (and often reproductively isolated) ecomorphs or species specialize on alternative resources within the same geographic location (i.e., lake). As a result the different morphs become infected mostly or exclusively with parasites acquired from the corresponding prey items. Phenotypic variation in feeding behavior arising from ontogenetic niche shifts (Knudsen et al. 1997; Amundsen et al. 2003) and differences between the sexes (Reimchen and Nosil 2001; Luong et al. 2013) may also introduce variation in exposure among individuals within a single population.

However, there often exists substantial phenotypic variation between individuals in traits such as habitat use or diet choice that is not accounted for by differences in age, sex, or by discrete polymorphisms (Bolnick et al. 2003). Even within an age class, sex, or morph, individuals vary in behavior and morphology in ways that lead to divergent resource use and consequently predictable variation in exposure to parasites among individuals (variation we refer to hereafter as phenotype-dependent exposure). In contrast to ecomorph- and sex-dependent parasitism, there is limited evidence for individual phenotype-dependent exposure and parasite infection in the wild. Johnson et al. (2009) showed that within a single population of sea otters (*Enhydra lutris nereis*) on the central California coast, individuals consuming more marine snails were more likely to be infected with the protozoan *Toxoplasma gondii*. Similarly, using polymerase chain reaction–based analyses, Luong et al. (2013) showed that white-footed mice (*Peromyscus leucopus*) were more likely to be parasitized by nematodes if they had consumed crickets (the intermediate host) in the recent past. However, neither of these studies attempted to link parasite infection directly to phenotypic variation. To our knowledge, only one study directly measured associations between parasite infection and individual phenotypic variation. Within a single sampling zone (littoral) of arctic charr (*Salvelinus alpinus*), individuals with more benthic morphology and more benthic prey in stomach contents were more likely to carry parasites transmitted through benthic prey (Knudsen et al. 2010). However, the relative contributions of habitat (limnetic- vs. benthic-caught fish) and individual variation were not statistically separated, and the study was not replicated across multiple populations.

Although it should be expected that individual variation in host phenotype should lead to differences in infection within single populations, it remains unclear how such phenotype-dependent exposure should contribute to among-population variation in infection. Among population variation in infection is often positively correlated with exposure among populations. However, positive correlations between exposure and infection are not necessarily the only possible pattern, as eco-evolutionary feedbacks can decouple these processes. For instance, populations that specialize on eating an intermediate host species might suppress the abundance of that prey, leading to reduced encounter rates with any parasites transmitted via the preferred prey. Alternatively, theory and intuition suggest that highly exposed populations may evolve more effective mechanisms to reduce infection (i.e., by evolving resistance) relative to populations with little or no exposure (Jokela et al. 2000; Zuk and Stoehr 2002; Schmid-Hempel and Ebert 2003). A number of studies have shown that resistance to infection and/or investment in immune defense is higher in populations exposed to parasites compared to those that are mostly or completely unexposed (Lindström et al. 2004; Kalbe and Kurtz 2006; Bryan-Walker et al. 2007; Hasu et al. 2009; De Roij et al. 2010; Eizaguirre et al. 2012). For example, Bryan-Walker et al.

(2007) showed that resistance to infection by trematodes in amphipods is higher in populations with high infection than in completely uninfected (no exposure) populations. In many of these cases, comparisons were made between exposed and unexposed populations, and thus, despite increased resistance in exposed populations, natural infection levels still correlate positively with exposure across populations.

In contrast, exposure can vary continuously from high to low, such as when host populations are on average shifted towards phenotypes that increase or decrease exposure risk. In such a situation, it is possible that increased resistance in more highly exposed populations could suppress infection to such a degree that natural infection rates could be decoupled from, or even covary negatively (rather than positively) with exposure rates. Such situations would be analogous to the phenomenon of countergradient variation (Conover and Schultz 1995). Evidence for such a pattern is lacking, possibly because of the difficulty of independently inferring infection and exposure rates across populations. Consequently, it is not clear as to whether within-population relationships between phenotype and infection can be extrapolated to among population comparisons or whether such relationships might be negated or reversed across larger spatial scales.

In this article, we address two questions using a large sample of a freshwater fish, the threespine stickleback (*G. aculeatus*) from 18 neighboring populations. First, using well-studied associations between stickleback morphology and diet, we ask whether individual morphology correlates predictably with the measured infection rate within populations. We use stickleback trophic morphology as a proxy measurement for exposure rate (which we cannot measure directly). Second, we ask whether these within-population associations between phenotype and infection can be extended across populations, by measuring the correlation between population mean phenotype and infection levels across populations. To our knowledge, this is the first such study to link phenotype-dependent exposure variation within populations to the expected relationship between exposure and infection among populations. Our results suggest that, although individual phenotype can be an important determinant of infection within populations, this relationship does not extend to among-population comparisons. We address potential explanations for these results, including the potential for the evolution of increased resistance, in "Discussion."

Study System

We tested for phenotype-dependent parasitism within and among populations of threespine stickleback (*Gasterosteus aculeatus*). In particular, we focused on infection stemming from variation in prey use among individual stickleback. Like many animal populations (Bolnick et al. 2003), stickleback in freshwater lakes exhibit substantial among-individual variation in diet (Schluter and McPhail 1992; Svanbäck and Bolnick 2007; Araújo et al. 2008; Bolnick and Paull 2009; Matthews et al. 2010; Bolnick and Araújo 2011). Although stickleback on the whole are generalist predators, within populations some individuals tend to consume more benthic macroinvertebrates, whereas others consume more limnetic zooplankton, and the degree to which individuals specialize on diet subsets is often dependent on the degree of intraspecific competition (Bolnick 2004; Svanbäck and Bolnick 2005, 2007; Araújo et al. 2008) and interspecific competition (Bolnick et al. 2010). This diet variation parallels, but is much more continuous than, the well-known ecological differentiation between sympatric benthic and limnetic species pairs of sticklebacks found in a few lakes (Schluter and McPhail 1992; Schluter 1993, 1995). The vast majority of lakes contain only a single species of stickleback with no discernible genetic or phenotypic subdivision of individuals (Lavin and McPhail 1985; Schluter 1993; Bolnick and Lau 2008), and neither phenotype nor genotype are bimodally distributed, nor is there a sign of within-lake genetic clusters (Caldera and Bolnick 2008).

We hypothesized that among-individual diet variation should confer different rates of exposure to parasites obtained by consuming benthic or limnetic intermediate hosts. Rather than directly assess prey use via gut content analysis, we used a set of continuous morphological traits as a proxy for prey use. Multiple morphological traits in stickleback are correlated with subtle but significant among-individual variation in the relative use of benthic versus limnetic prey, as revealed by studies of stable isotope ratios (Snowberg and Bolnick 2008; Matthews et al. 2010; Bolnick and Araújo 2011) and cross-sectional analysis of gut contents (Schluter and McPhail 1992; Svanbäck and Bolnick 2007; Araújo et al. 2008; Bolnick and Paull 2009). We focus on three traits commonly and strongly associated with diet within stickleback populations—body size, relative gill raker length, and gill raker number—as our phenotypes of interest (Schluter 1993; Robinson 2000; Araújo et al. 2008; Bolnick and Lau 2008; Matthews et al. 2010; Bolnick and Araújo 2011). More benthic diets are typically associated with larger fish that have fewer and shorter gill rakers, whereas more limnetic diets are typically associated with smaller fish that have longer and more numerous gill rakers. We note that, for present purposes, using morphology as a proxy for diet choice is preferable over instantaneous diet snapshots because the latter reflect diet only in the few hours before capture, whereas parasite load will depend on long-term

Table 1: Samples sizes, percent infected, and mean morphology scores by lake

			Fish sampled			% infected (nematodes)			% infected (cestodes)			Mean morphology scores		
Lake	Year	Watershed	Total	Females	Males	Total	Males	Females	Total	Males	Females	Body size	rGRL	rGRN
Amor	2005	Amor	101	48	53	21.1	21.7	20.4	18.3	25.0	10.2	−2.33	.15	.05
Blackwater	2005	Amor	497	309	188	10.6	9.0	11.7	2.0	1.1	2.6	−.42	−.12	.03
Cecil	2006	Amor	400	176	224	1.8	1.3	2.3	.8	.4	1.1	.20	−.18	−.07
Cedar	2005	Amor	243	109	134	10.7	11.2	10.1	3.7	4.5	2.8	−1.19	−.09	.09
Comida	2005	Mohun	162	71	91	2.5	1.1	4.2	.6	1.1	.0	1.21	−.02	.06
Farewell	2005	Amor	300	163	137	2.0	1.5	2.5	3.7	1.5	5.6	.21	−.11	.02
First	2005	Salmon	496	231	265	3.4	1.9	5.2	5.8	5.7	5.6	.24	−.07	.00
Gosling	2006	Campbell	399	244	155	1.5	1.9	1.2	76.3	60.6	86.5	1.24	.03	−.01
Gray	2006	Campbell	400	208	192	1.7	2.6	1.0	.2	.0	.5	1.68	.12	−.02
Little Mud	2006	Amor	400	289	111	1.0	1.8	.7	2.5	.9	3.1	.45	−.07	.03
Little Woss	2005	Woss	300	164	136	2.0	.7	3.0	19.0	22.1	16.5	1.36	.08	.01
McCreight	2005	Amor	500	345	155	21.4	20.6	21.7	.0	.0	.0	−.57	.08	−.05
McNair	2006	Amor	400	127	273	.3	.0	.8	4.5	3.7	6.3	.65	−.03	.08
Mohun	2006	Mohun	399	163	236	2.5	3.9	.6	5.8	5.6	6.2	.28	.08	.06
Mud	2006	Amor	397	277	120	4.5	4.2	4.7	1.3	1.7	1.1	1.30	−.02	.05
Roberts	2005	Amor	530	274	256	7.1	5.5	8.8	.0	.0	.0	−.74	−.05	−.05
Roberts	2006	Amor	400	212	188	21.5	18.1	24.5	.0	.0	.0	−1.55	−.10	−.06
Second	2006	Salmon	399	172	227	1.5	1.8	1.2	2.3	3.1	1.2	−1.84	.17	−.03
Snow	2005	Pye	476	286	190	1.3	1.6	1.0	.2	.0	.3	−.18	.17	−.04

Note: Mean body sizes are the average score on the first principal component of morphological variation (see "Material and Methods "). rGRL = relative gill raker length (mm); rGRN = relative gill raker number. Larger relative values of rGRL and rGRN indicate populations with longer and more numerous gill rakers, controlling for body-size variation. UTM coordinates for each lake are given in table A1, available online.

diet history (Reimchen and Nosil 2001; Knudsen et al. 2011).

Although stickleback tend to be infected with a wide variety of trophically transmitted parasites (Wootton 1977; Kalbe et al. 2002; De Roij and MacColl 2012), we focus on two common helminth parasites of stickleback in our study area: the cestode *Schistocephalus solidus* and a nematode in the genus *Eustrongylides*. Although it is often the case that multiple parasites show correlations with diet (i.e., Knudsen et al. 2010), these two parasites have the advantage of being easily identified and counted in thousands of fish (when the body cavity is opened), without the need to for laborious microscopic examination to obtain accurate counts. Although both parasites use stickleback as a second intermediate host to reach a definitive host (predatory birds), *S. solidus* are acquired by stickleback from feeding on infected cyclopoid copepods (a limnetic resource) whereas *Eustrongylides* sp. are acquired from benthic oligochaetes.

We predicted that more benthic individuals (i.e., larger with relatively shorter and less numerous gill rakers) were more likely to be infected with *Eustrongylides* sp., while more limnetic fish (smaller, more numerous, and relatively longer gill rakers) would more likely be infected with *S. solidus*. By extension, we predicted that populations that were on average more benthic (limnetic) in phenotype would have higher rates of infection of *Eustrongylides* spp. (*S. solidus*). Alternatively, the opposite relationship between mean phenotype and infection would indicate additional eco-evolutionary processes are swamping the role of exposure in generating among-population variation in infection rates.

Material and Methods

Stickleback were collected during the months of June and July in 2005 and 2006 from 18 lakes on northern Vancouver Island, British Columbia (tables 1, A1; tables A1–A8 available online). The sampled lakes are contained within six neighboring watersheds that share a recent geological history, and gene flow is limited between neighboring lakes (Caldera and Bolnick 2008). Mean sample size was 381 fish per lake. Fish were captured and morphologies measured as described in Bolnick and Lau (2008).

We focused our analysis on three morphological traits that are typically the strongest predictors of within-population diet in lake stickleback—body size, gill raker length (of the longest gill raker), and gill raker number (Robinson 2000; Araújo et al. 2008; Matthews et al. 2010; Bolnick

and Araújo 2011). These traits are unimodally distributed within our populations (e.g., the populations are not composed of distinct benthic and limnetic morphs, rather individuals vary continuously between benthic-like and limnetic-like extremes). We size standardized both gill raker length and gill raker number using the method advocated by Berner (2011). After pooling all samples, a principal components analysis was performed on all log-transformed morphological traits. All traits (except gill raker number which is uncorrelated with size) loaded positively on the first principal component axis (accounting for 63% of total variation). The principal component scores on this first axis were used as our measure of individual body size. Both gill raker length and gill raker number (all samples pooled) were subsequently regressed on body size to obtain residuals representing size-standardized morphological traits (hereafter relative gill raker length [rGRL] and relative gill raker number [rGRN]). Standardizing variables within each lake produced highly similar results (data not shown). Body size, relative gill raker number, and gill raker length were scaled to have a mean of zero and unit variance before further analysis.

Each fish was dissected and the numbers of *Schistocephalus solidus* and *Eustrongylides* sp. were recorded. Sex was determined by inspecting gonads. For each lake, the prevalence (proportion of infected fish) and mean parasite abundance (average number of parasites per fish) were calculated (tables 1, A1). We estimated the correlation in prevalence of the two parasites across lakes using standard Pearson regressions of both the raw and logit-transformed prevalence data (lakes with zero prevalence were removed from the logit-transformed data set). We used χ^2 tests within each lake population to determine whether coinfection with the two parasite species occurred at lesser or greater rates than expected by chance, and we corrected for multiple tests using the method of Hochberg (1988). All data from this study are deposited in the Dryad Digital Repository (http://dx.doi.org/10.5061/dryad.653c9; Stutz et al. 2014).

Statistical Analyses: Does Individual Phenotype Covary with Individual Infection Status?

We used generalized linear mixed models (GLMMs) to model infection as a function of individual morphology and sex. Both the probability of infection (binomial data) and the number (abundance) of parasites per fish (count data) were initially used as response variables. Because our results were similar with both measures, and because the majority of parasitized fish were parasitized with only one of either parasite, we report only the results for the probability of infection. Nematode and cestode infection were analyzed separately. We used logit link functions in all models.

We initially included each morphological variable (body size, relative gill raker length, relative gill raker number), sex, and the interaction between sex and each morphological variable as potential fixed effects (seven total). Following Zuur et al. (2011), we next identified the optimal random-effects structures for our GLMMs. A lake intercept term accounting for variation in the probability of infection among lakes was included as a random effect in all models. Both year of collection and watershed were originally considered as potential random effects, but preliminary analyses indicated that they did not improve model fit and these effects were left out of all subsequent model comparisons (table A2). We also initially included additional random slope terms for each morphological trait and for sex to account for potential variation in these effects among lakes (as could occur, for example, if morphology or sex is more tightly correlated with diet in some populations than in others). These terms are analogous to fixed-effects interaction terms between lake and morphology, except that only the variance introduced by the interactions is estimated and accounted for. The overall random-effects structure that produced the lowest AIC value among compared structures was considered optimal and was used subsequently to estimate the fixed effects (Zuur et al. 2011). If random-effects structures included correlations among random effects (i.e., between lake intercept and the effect of body size), these correlations were tested for inclusion in the model by removing the estimated correlation and testing the nested models using log-likelihood ratio tests.

To determine which fixed effects should be included in each model, we used a step-down approach to remove fixed interaction terms with the smallest Wald z values. Nested models were compared using log-likelihood ratio tests. We opted to retain all noninteraction fixed effects in the reported models in order to compare the magnitude and direction of the estimated fixed effects to our a priori predictions for each trait. All GLMMs were fit using maximum likelihood as implemented in the package lme4 (Bates et al. 2011) in the statistical programming environment R (R Core Team 2012).

In addition to removing nonsignificant interaction terms, we also used a parametric bootstrap approach to calculate P values for each fixed effect retained in the final models. Specifically, we simulated infection levels for each lake by drawing random samples from a binomial distribution parameterized using the prevalence calculated for each lake. We then refitted the simulated data set to the optimal GLMM determined above. We ran 1,000 bootstrap simulations per model. Two-tailed P values for each fixed effect were calculated by comparing our effects size esti-

mates to the simulated null distribution. Analyses using Bayesian Markov chain Monte Carlo methods using the package MCMCglmm (Hadfield 2010) produced very similar results (not shown). The *P* values calculated from bootstraps and log-likelihood ratio tests were in agreement about which fixed effects were statistically significant.

How Does Population Mean Phenotype Covary with Parasite Prevalence?

We used GLMs with quasi-binomial error structure and logit links to predict the proportion of fish parasitized (prevalence) as linear and quadratic functions of the mean of each morphological trait for each lake. Roberts Lake was treated as a single large collection across both years, which in effect averages the results for the two years, albeit with a large sample size. Models were fit using the glm function in the base stats package in R.

Because we were interested in whether the within-population associations between phenotype and infection would be recapitulated among populations, we tested whether the estimated among-population effect of each of morphological variable (from the GLMs) was significantly different than the corresponding among-individual effect (from GLMMs) using Welch's *t*-test (i.e., for samples with unequal variances).

Results

Overall Rates of Parasitism

Nematode prevalence ranged from 0.3% to 21.5% of fish per lake (table 1). Cestode prevalence ranged from 0% to 76.3%, although all but one lake had less than 20% prevalence (table 1). The outlier (Gosling Lake) has sustained this high prevalence of *Schistocephalus* for more than a decade (D. Bolnick, personal observation). There was no correlation in the prevalence of nematodes and cestodes across lakes using raw prevalences ($r = -0.08$, $P = .76$) or logit transformed prevalences ($r = 0.13$, $P = .64$). One of 19 lakes contained more coinfected fish than expected by chance, (Mohun Lake, $\chi^2 = 6.83$, $P = .009$) while one other lake had significantly fewer fish coinfected than expected (Amor Lake, $\chi^2 = 5.44$, $P = .019$). Neither of these tests were significant when controlling for multiple comparisons. Thus, infection with one parasite had no measurable association with the probability of infection with the other parasite within lakes, and we do not account for joint infection in the subsequent analyses.

Nematodes: Does Individual Phenotype Correlate with Infection Status?

For all results, regression coefficients are the estimated change in log odds of infection per unit of the corresponding phenotype. Overall, larger fish (a benthic trait) were more likely to be infected with benthic-derived nematodes ($\beta_{\text{SIZE}} = 0.16$, $P < .001$, fig. 1*A*). This trend is consistent with our model prediction of a positive correlation between individual phenotype and infection rate. Neither relative gill raker length or number were significantly associated with the probability of nematode infection among individuals overall ($\beta_{\text{rGRL}} = 0.63$, $P = .66$; $\beta_{\text{rGRN}} = -0.73$, $P = .34$, fig. 1*B*, 1*C*). Controlling for the effects of morphology, females were on average 1% more likely to have a nematode than males ($\beta_{\text{SEX}} = -0.32$, $P < .001$).

The optimal model included a term for variation in the effect of relative gill raker length among lakes ($\sigma^2_{\text{rGRL}} = 2.82$, table A3), indicating that the effect of relative gill raker length on the probability of nematode infection varied substantially among lakes (fig. 1*B*). This variation in the relative gill raker length effect was also strongly negatively correlated with the intercept term (i.e., the prevalence of nematodes) for each lake ($r = -0.84$, $\chi^2 = 4.31$, $P = .028$). This negative correlation indicates that, in lakes with a higher rates of nematode infection, shorter-rakered fish (more benthic) were increasingly more likely to parasitized with benthic nematodes (dotted lines, fig. 1*B*). However, because in many lakes nematode prevalence was near zero, the overall effect of relative gill-raker length across lakes was negligible and not significantly different than zero. That is, a biologically real effect of morphology within some lakes is obscured in the overall model by among-lake heterogeneity in morphology-dependent infection.

Nematodes: Does Population Mean Phenotype Covary with Prevalence?

The correlation between individual morphology and nematode infection status does not extrapolate to among-population comparisons. Paradoxically, the benthic nematode was more prevalent in morphologically more limnetic populations. Nematode prevalence was significantly higher in lakes with smaller (more limnetic) fish ($\beta_{\text{SIZE}} = -0.56$, $P = .03$, fig. 1*D*) and marginally significantly higher in lakes with relatively longer-rakered fish ($\beta_{\text{rGRL}} = 3.75$, $P = .08$, fig. 1*E*). Relative gill raker number showed no relationship with nematode infection among lakes ($\beta_{\text{rGRN}} = 0.86$, $P = 0.87$, fig. 1*F*). No quadratic effects of mean morphology on prevalence were found ($\gamma_{\text{SIZE}} =$

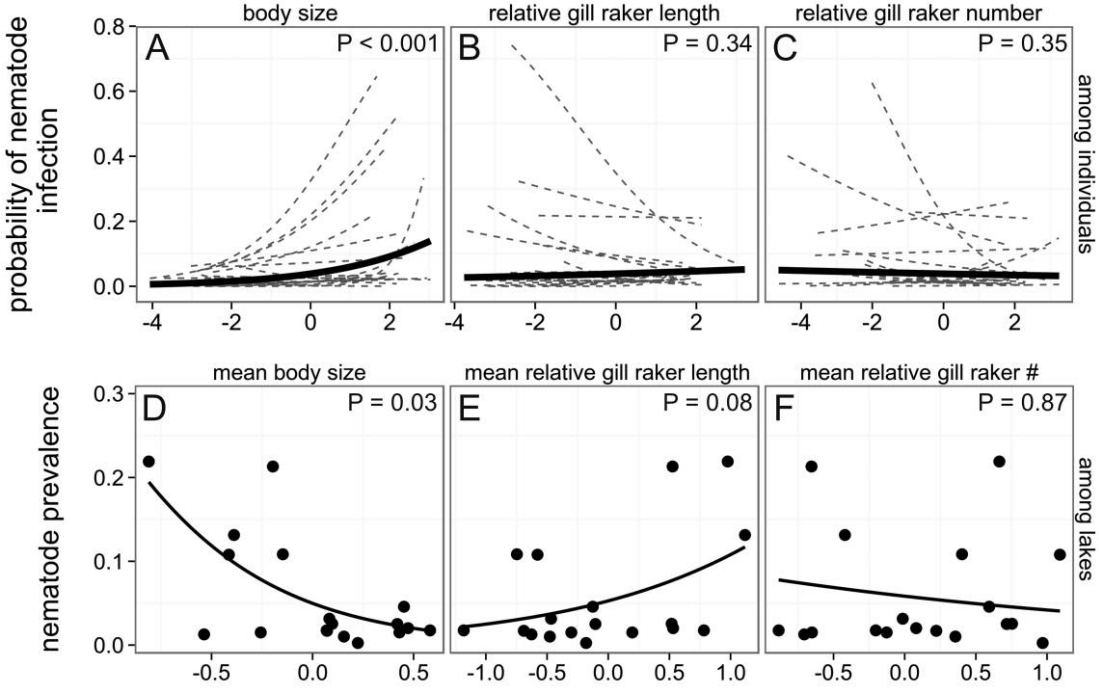

Figure 1: *A–C*, Relationships between the probability of nematode infection and phenotypes among individuals. Each phenotype was scaled to unit variance before analysis. Solid lines indicate the estimated regression parameter for each independent variable from the global generalized linear mixed model, which indicates the overall relationship between morphology and infection accounting for lake-to-lake differences. The gray dotted lines in each panel indicate the same estimated regression coefficient within each lake individually. *D–F*, Relationships predicting nematode prevalence as a function of mean phenotypes, with each point representing values from a single lake. Mean phenotypes in *D–F* correspond to the same phenotypes in *A–C*, respectively. Single-factor regressions are plotted for each morphological variable; however, *P* values refer to the significance of the partial regression coefficients (table A5, available online).

-0.16, $P = .53$; $\gamma_{rGRL} = 20.1$, $P = .44$; $\gamma_{rGRN} = 18.0$, $P = .91$).

The effects of body size and relative gill raker length on the probability of nematode infection were significantly different between individual and population scales (i.e., comparing overall effects from fig. 1*A*–1*C* vs. fig. 1*D*–1*F*; size: $t = 12.9$, df $= 17$, $P < .0001$; rGRL: $t = 6.7$, df $= 17$, $P < .00001$), but not so for relative gill raker number (rGRN: $t = 1.35$, df $= 17$, $P = .10$). Thus, the observed increases in the probability of infection with both larger size and shorter gill rakers among individuals were not recapitulated when comparing infection levels to the mean values of these two traits among lakes. No such difference was indicated for gill raker number.

Cestodes: Does Individual Phenotype Correlate with Cestode Infection Status?

As with nematodes, cestode trends were consistent with our model prediction of a positive correlation between morphology and infection within populations. More limnetic fish (smaller and longer gill rakers) were more likely to be parasitized ($\beta_{SIZE} = -0.16$, $P < .001$; $\beta_{rGRL} = 1.32$, $P < .001$; β_{rGRN}: $\beta = -1.48$, $P = .13$, fig. 2*A*–2*C*). Controlling for individual differences in morphology, males were no more or less likely to be parasitized than females ($\beta_{SEX} = 0.03$, $P = .97$). However, females showed a significantly larger increase in infection probability with smaller body size than males ($\beta_{SIZE \times SEX} = 0.11$, $P < .001$). Refitting the model to each sex separately revealed that the predicted body size effect was significant in females ($\beta_{SIZE} = -0.17$, $P < .001$) but not in males ($\beta_{SIZE} = -0.02$, $P = .69$).

The optimal model also included variation in the random sex effect ($\sigma^2_{SEX} = 0.39$, table A4), indicating that there was substantial variation in the effect of sex on cestode infection across lakes. This variation was negatively correlated with lake intercept term ($r = -0.63$, $\chi^2 = 12.7$, $P = .0004$), indicating that in lakes with higher cestode infection, females had increasingly larger probabilities of being infected relative to males. However, this result was dependent on the inclusion of Gosling Lake (which had both the highest cestode prevalence by far and also the greatest disparity between male and female infection rates;

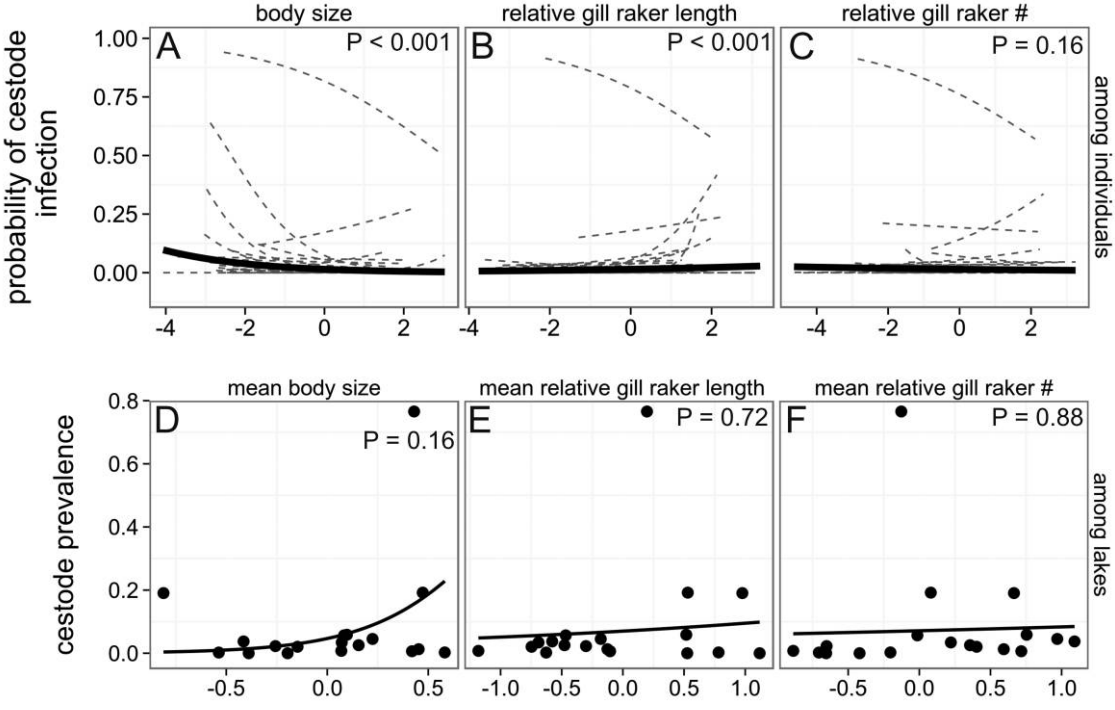

Figure 2: *A–C*, Relationships between the probability of cestode infection and phenotypes among individuals. Each phenotype was scaled to unit variance before analysis. Solid lines indicate the estimated regression parameter from the global generalized linear mixed model, which indicates the overall relationship between morphology and infection accounting for lake-to-lake differences. The gray dotted lines in each panel indicate the same estimated regression coefficient within each lake individually. *D–F*, Relationships predicting cestode prevalence as a function of mean phenotype, with each point representing values from a single lake. Mean phenotypes in *D–F* correspond to the same phenotypes in *A–C*, respectively. Single-factor regressions are plotted for each morphological variable; however, *P* values refer to the significance of the partial regression coefficients (table A5, available online).

table 1). Excluding this lake, a refit model did not support the inclusion of the random sex effect, indicating there was no additional heterogeneity in relative probabilities of male and female infection among lakes.

Does Population Mean Phenotype Covary with Cestode Prevalence?

Despite significant within population associations, neither body size, rGRL, or rGRN were significantly correlated with overall cestode prevalence ($\beta_{SIZE} = 1.07$, $P = .20$, $\beta_{rGRL} = 1.11$, $P = .88$, $\beta_{rGRN} = -4.29$, $P = .79$, fig. 2*D–2F*). No significant quadratic effects of mean morphology on prevalence were found ($\gamma_{SIZE} = 0.24$, $P = .69$, $\gamma_{rGRL} = -227.4$, $P = .28$, $\gamma_{rGRN} = -647.5$, $P = .20$).

The estimated individual level and population level slopes were significantly different only for body size (body size: $t = 6.5$, df $= 17$, $P < .0001$; rGRL: $t = 0.12$, df $= 17$, $P = .45$; rGRN: $t = 0.78$, df $= 17$, $P = .24$). Thus, the observed significant increase in the probability of infection with decreased size among individuals was not recapitulated when comparing infection levels to mean body size among lakes. In contrast, gill raker effects among populations were commensurate with gill raker effects within populations.

Discussion

It is intuitive that individual phenotypes conferring increased exposure to a parasite should be correlated with infection, even in populations without discrete phenotypic polymorphisms. However, there is no guarantee that populations generally contain enough phenotypic variation to generate measurable correlations. We showed, across 18 populations of stickleback, that the probability of infection with the benthic nematode *Eustrongylides* sp. increased for fish with more benthic phenotypes, while contrastingly, the probability of infection with *Schistocephalus solidus* (acquired by eating limnetic prey) increased in fish with more limnetic phenotypes. This was true despite the fact that all fish were caught in the same habitat in each lake and show absolutely no evidence of discrete polymorphism

within any populations (e.g., no distinct benthic or limnetic morphs within any population). These results mirror findings from single or paired populations in species showing discrete resource use polymorphisms (Bertrand et al. 2008; Knudsen et al. 2008, 2011; MacColl 2009; Karvonen et al. 2013) and studies of morphologically undifferentiated populations of stickleback and other species where heterogeneities in diet related exposure have been attributed to spatial, behavioral, sexual, and/or individual differences (Reimchen and Nosil 2001; Yohannes et al. 2008; Johnson et al. 2009; Knudsen et al. 2010; Parker et al. 2010; Luong et al. 2013). Our results represent one of only a few studies to test for effects of individual foraging differences on infection rates in unimodal populations and the first to show that these effects hold across many populations concurrently. Our results also imply that variation in exposure, and thus infection, among individuals is a likely common ecological consequence of individual specialization.

Although our predictions for stickleback are based on diet-mediated exposure, host ontogeny and life-history effects could play roles in generating the within-population patterns of infection we see here. Older (and thus larger) fish could have more parasites simply by being exposed over a longer period of time, or by eating more food, than younger fish (Poulin 2000). Neither hypothesis could explain why smaller fish showed a higher probability of being infected with cestodes or why populations with smaller mean size had more nematodes. Alternatively, a lower probability of cestode infection in larger fish could potentially be explained by die-off of heavily infected hosts (Crofton 1971). However, both rGRL and rGRN are body-size independent and also show the trends predicted by morphology-diet correlations for cestodes, indicating the body size trends also likely result from the same diet-parasite correlations.

Rather than morphology influencing the probability of infection via increased exposure, it is also possible that infection alters the morphology of hosts directly, thus reversing our proposed cause and effect. More specifically, our within-lake patterns could potentially be explained if infection with *S. solidus* reduced body size and increased relative gill raker length (and *Eustrongylides* increased body size and reduced relative gill raker length) in infected fish. In stickleback, previous observational and experimental studies have shown that *S. solidus* infection often (thought not always) results in reduced body mass and body condition but not in reduced standard length (Barber and Svensson 2003; Barber et al. 2008). Moreover, our body size measurement was a composite score based on morphological traits that should be relatively unaffected by changes in relative body mass or condition (i.e., total length, gill raker length, lower jaw length, dorsal spine length, etc. See Bolnick and Lau 2008 for a full list of traits). Moreover, the population with by far the highest prevalence of *S. solidus* (~80%) also had one of the largest mean body sizes among our studied populations (fig. 2*A*), suggesting that *S. solidus* does not substantially reduce growth rate in our system. Given the observed harmful physiological effects of *Eustrongylides* in other fish systems (Paperna 1974), it also seems unlikely that *Eustrongylides* infection would lead to increases in individual body size, although we cannot rule out such a possibility. We believe, therefore, it is unlikely that either parasite is having a strong effect on either our body size (or gill raker) traits, and that the within-lake patterns are most parsimoniously explained by differences in diet-based exposure.

Given that individual infection was correlated with trophic phenotype, we were able to empirically evaluate whether the same correlations were observed across populations using mean phenotypes. We found that among-population variation in prevalence was generally not affected by phenotype in the same direction as predicted from among individual differences. In the case of the nematode parasite, both mean body size and mean relative gill raker length were negatively associated with nematode prevalence among lakes (fig. 1*D*, 1*E*), indicating that phenotypically more limnetic populations had higher rates of benthic nematode infection. For cestodes, infection did not vary positively or negatively with population mean phenotype, despite the fact that individual-level infection very clearly showed that more limnetic individuals were more likely to be infected. Overall, the within-population trends were not recapitulated among populations. There are a number of possible explanations for this countergradient pattern.

First, although our lakes are relatively similar overall, it is possible that baseline (i.e., non-phenotype-dependent) exposure could vary among populations. Such variation in exposure could arise because of variation in the density of intermediate hosts within populations, the rate at which those intermediate hosts are infected, and the rate at which infected hosts are consumed by stickleback. Zooplankton (and specifically copepod) abundance covaries with lake geomorphology such that larger, deeper lakes have higher zooplankton densities than smaller shallower lakes (tables A6, A7). Such lakes also generally have morphologically more limnetic stickleback (Berner et al. 2010). If anything these observations should reinforce the prediction that large, deep lakes with limnetic populations should be more heavily infected with the limnetic *S. solidus*—a pattern rejected because of the data presented here. Moreover, neither total zooplankton density nor copepod density correlates significantly with the prevalence of either parasite across lakes (table A8). Variation in the density of the first intermediate host of nematodes (oligochaetes) in our sys-

tem is currently unknown, although we can propose no plausible explanation for why there would be a negative covariance between oligochaete density and benthic morphology in stickleback across populations. In one other large study of parasite abundance among neighboring lakes of threespine stickleback in Scotland, De Roij and MacColl (2012) found no effect of lake geomorphology or four physiochemical variables on the abundance of any parasite species among lakes, suggesting these features may not be important determinants of parasitism in stickleback among neighboring lake populations.

Similarly, we do not know whether infection rates among first intermediate hosts could be driving the observed variation in prevalence among lakes. Infection rates in intermediate hosts are typically uniformly very low in freshwater invertebrate hosts (Marcogliese 1995; Knudsen et al. 2001), and fish likely accumulate parasites by eating very large quantities of very sparsely parasitized prey (Marcogliese 1995). It is possible that the terminal hosts for our parasites prefer lakes with a certain mean phenotype of fish, preferentially depositing infectious stages in those lakes (Hechinger and Lafferty 2005). Given that both parasites investigated here are transmitted by piscivorous birds, this factor alone would likely not explain why the prevalences of the two parasites are uncorrelated among populations. Moreover, in 15 years of work on these lakes, we have observed no differences in piscivorous bird abundance among these lakes (D. Bolnick, personal observation).

It has been suggested that hosts could be selected to actively avoid parasitized prey (Lozano 1991; but see Lafferty 1992). The evolution of such behavior in highly exposed populations could be classified as an evolved "resistance" trait, broadly speaking (Schmid-Hempel and Ebert 2003) and thus account for the among population trend in our data (i.e., lower infection in more exposed populations). However, evidence for such behavioral variation is lacking, including studies done in stickleback tested with infected and uninfected copepods (Wedekind and Milinski 1996; Barber et al. 2000). It may be the case that the energetic rewards of certain prey outweigh either the costs of evolving effective discrimination behaviors or the potential fitness effects of being infected (Lafferty 1992). Taken altogether, the likelihood that baseline exposure rates for the benthically derived nematodes are negatively correlated with more benthic mean morphologies across populations seems unlikely, although we cannot rule this possibility out at present.

Although we focused on *S. solidus* and *Eustrongylides* for practical and ecological reasons (see "Introduction"), stickleback populations often contain a diverse array of macroparasite species (Andersen and Valtonen 1992; Kalbe et al. 2002; MacColl 2009). Infections with other parasites could alter patterns of *S. solidus* and *Eustrongylides* infection in at least two ways. First, other parasites can directly alter the survival of coinfected individuals. For example, stickleback are often infected with trematodes of the genus *Diplostomum* (i.e., eye flukes), which can impair vision and increase mortality, presumably by increasing predation risk (Lester 1971; Owen et al. 1993; McKeown and Irwin 1997). Heavy *Diplostomum* infections could influence the patterns of infection observed in the present study by reducing the presence of individuals also infected with *S. solidus* or *Eustrongylides*. Second, infection with another parasite could alter host immune status, preventing (or facilitating) subsequent infection with *S. solidus* or *Eustrongylides* (Ezenwa et al. 2010; Telfer et al. 2010; Johnson et al. 2013). Of course, the extent to which other parasites are likely to alter the correlation between morphology and cestode or nematode infections is less clear but will presumably depend on whether the other parasite is itself correlated with host morphology within or among host populations. More focused studies in one or a few populations could determine to what degree other species cooccur with our focal species and contribute to the patterns of variation we see here. However, at this point the contribution of other parasites in our results remains an open question

Perhaps the most intriguing explanation for our countergradient pattern, alluded to in "Introduction," is that populations that are highly exposed to each parasite would evolve enough resistance (or reduce susceptibility in some other way) to substantially reduce overall infection levels when compared to populations with lower exposure where selection pressures may not be as strong (Jokela et al. 2000; Zuk and Stoehr 2002; Schmid-Hempel and Ebert 2003; Hasu et al. 2009). How this could lead to the countergradient trends in our nematode data (or any comparable data set) is illustrated in figure 3. To start, phenotypically benthic individuals are more exposed to nematodes than limnetic individuals due to greater consumption of benthic prey. This leads to a positive correlation between individual phenotype and infection levels and between population mean phenotype and population mean infection levels (fig. 3*A*, large solid line). Over time, high exposure leads to the evolution of reduced susceptibility via increased resistance to nematodes in more benthic populations, allowing fish in those populations to prevent nematode infection and leading to a decreased infection levels (indicated by downward arrows in fig. 3*B*). In contrast, limnetic populations, lacking resistance, would have higher infection levels despite relatively lower exposure. Over time, this process would lead to negative correlation between mean phenotype and infection rate among populations, consistent with our data (solid line, fig. 3*C*). Moreover, despite the evolution of resistance at the population level, individuals

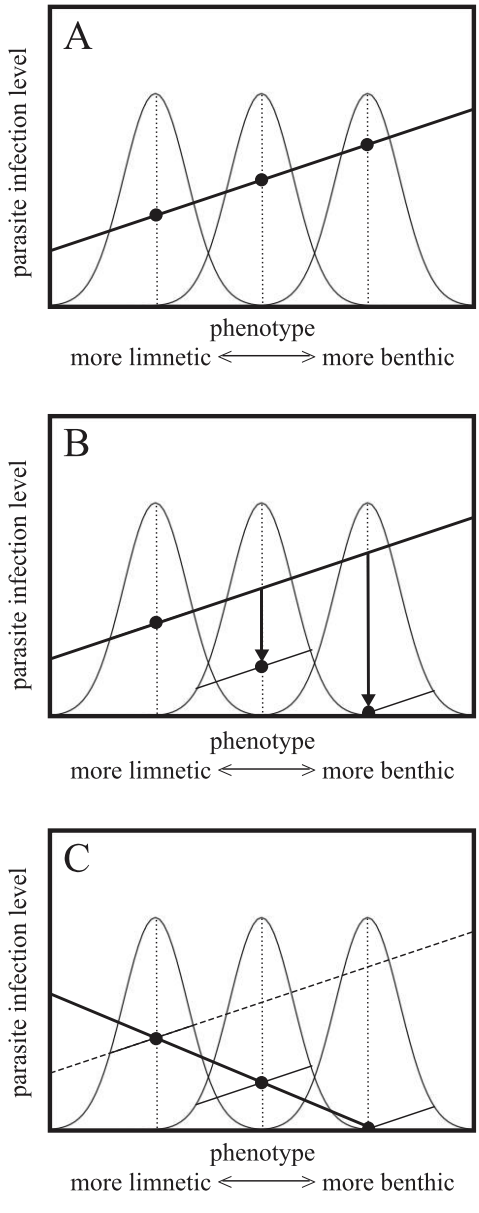

Figure 3: An illustration of how evolved resistance—in response to high exposure—can account for the among-population trends in our data (and other comparable data). On the *X*-axis is an arbitrary phenotypic measure that correlates positively with exposure to a given parasite (e.g., morphology). The *Y*-axis indicates some measure of infection (i.e., prevalence or abundance). In all panels, the curved lines indicate the phenotypic distributions of three hypothetical populations with phenotypic means indicated by the vertical dotted lines. Infection levels for each population are indicated by the solid black dots in each panel. *A*, Before any evolution, more benthic populations have both higher exposure and higher levels of infection than do more limnetic populations. *B*, Over time, high rates of exposure lead to the evolution of resistance in more benthic populations (indicated by downward arrows), decreasing infection levels more in benthic populations relative to limnetic populations. *C*, The final result is a negative correlation between population mean phenotype and infection level.

within populations should still show positive correlations between phenotype and load (indicated by shorter solid lines confined within the populations curves in fig. 3), which was also confirmed by our data. This hypothesis could also explain the flat relationship between mean morphology and cestode prevalence among lakes, if the evolution of decreased susceptibility is just strong enough to counterbalance variation in exposure (a straight line, rather negatively sloped solid line connecting phenotypic means, in fig. 3*C*).

The applicability of this hypothesis to our data assumes that resistance covaries with exposure (and thus morphology) at the among-population but not the among-individual level. Across populations isolated by low gene flow, correlated selection for resource use and resistance could readily build up this covariance. Within populations such correlations are less likely. There is some potential for linkage disequilibrium to build between the genes controlling both resistance and phenotype within populations if resistance is costly, because unexposed individuals would not be selected to bear potential costs of resistance (Sheldon and Verhulst 1996). Such linkage disequilibrium could be enhanced by nonrandom mating among individuals with similar morphologies or diet preferences (Snowberg and Bolnick 2008). However, of the populations we sampled, only one is known to be out of Hardy-Weinberg equilibrium at putatively neutral microsatellite markers spaced throughout the genome (Caldera and Bolnick 2008), indicative of random mating. Two other populations in the study area (not sampled here) show weak assortative mating by diet (Snowberg and Bolnick 2008, 2012). Most importantly, any strong linkage between resistance and phenotype within populations should obscure the predicted direct relationship between phenotype and exposure, a pattern contradicted by our empirical results.

A number of lines of additional evidence suggest that evolved resistance could account for the among-population pattern we found here. First, there is ample evidence from both lab and field studies that stickleback populations have evolved resistance to commonly encountered parasites when compared to populations in habitats where the same parasites are uncommon or absent (Kalbe and Kurtz 2006; Scharsack et al. 2007*a*; De Roij et al. 2010; Eizaguirre et al. 2012). Kalbe and Kurtz (2006) showed that lake ecotypes of stickleback that are naturally highly exposed to *Diplostomum* sp. trematodes in the wild show greater resistance to experimental infection with *Diplostomum* compared to river ecotypes, which are rarely or never naturally exposed. De Roij et al. (2010) showed a similar, but nonsignificant, trend in stickleback using the parasite *Gyrodactylus*. Using reciprocal transplants, Scharsack et al. (2007a) showed that lab-reared F_2 river ecotypes transplanted into lake enclosures had significantly greater par-

asite burden than did lake ecotypes transplanted into the same lake habitat, suggesting some degree evolved resistance in the ecotypes to the parasites present the two habitats. Additionally reciprocal transplants performed between a lake in our study (Roberts) and its outlet stream show a similar pattern of local adaptation, with stickleback becoming more infected with parasites in the contrasting environment to which they are not naturally exposed than do native fish (W. E. Stutz, unpublished data).

Second, immunological studies, particularly from the threespine stickleback/*Schistocephalus* system, suggest some possible mechanisms by which stickleback could decrease infection success of *Schistocephalus* (Barber and Scharsack 2010), and potentially other parasites such as nematodes which infect stickleback in a similar manner. These include increased enzymatic or digestive activity in the intestinal tract (Hammerschmidt and Kurtz 2007) and early recognition and upregulation of monocytes on exposure to cercariae (Barber et al. 2001; Scharsack et al. 2007*b*). The adaptive immune system has also been shown to play a role in stickleback adaptation to parasites in the wild. In a reciprocal transplant of lab-reared fish, Eizaguirre et al. (2012) showed that certain major histocompatibility genotypes (important for parasite recognition) common in river ecotypes were associated with increased resistance for a dominant river parasite (*Gyrodactylus* sp.). In contrast, lake ecotypes, which he naturally much lower mean abundances of *Gyrodactylus*, did not carry the same alleles and suffered increased rates of infection after transplantation into the river. These transplant studies suggest that exposure variation between different habitats has led to local adaptation of stickleback populations with respect to commonly encountered parasites and that both innate and acquired immunity can potentially underlie differences in parasite resistance. Both innate and acquired immunity could also potentially confer distinct immunological responses to the nematodes and cestodes studied here, though the potential for cross-immunity is unknown.

A third line of evidence for evolved resistance in response to high exposure comes directly from our data. Although at the individual level the probability of infection was positively correlated with morphology, the strength of the correlation was not uniform among lakes. Instead, benthic fish were more likely to be infected with nematodes (the benthic parasite) in overall more limnetic populations (cf. figure 1*A* and 1*D*). This trend can be clarified by noting that even within populations where the mean morphology is shifted toward the limnetic end of the spectrum, some individuals remain comparatively benthic. As our data indicates, it is this minority of relatively exposed benthic individuals, in otherwise limnetic-shifted populations, that become highly parasitized by nematodes. This result is consistent with the evolved resistance hypothesis, which predicts that while individual fish in benthic populations should be immunologically well adapted to frequently encountered benthic parasites, fish in limnetic populations will not be, and consequently relatively more highly exposed benthic individuals in limnetic populations will also be more highly infected. In other words, a mismatch between the population's immunological adaptations and the diet of a small minority of individuals could lead to the within-lake correlations between morphology and infection we see in the data.

In conclusion, we presented data from 18 populations of threespine stickleback and showed that infection was positively correlated with morphologies that increase exposure within populations. This is consistent with previous work showing similar correlations in populations with discrete trophic polymorphisms, but the first to show that individual phenotypic differences reliably predict infection across multiple, phenotypically unimodal, and ecologically undifferentiated populations. However, the data indicated that phenotype-dependent exposure differences between populations are not driving trends in infection at the population level. A number of possible mechanisms could potentially account for the observed variation in infection among populations, including variation in exposure independent of host phenotype. The data (especially from nematodes) are also consistent with a prediction of evolved resistance in response to high exposure, as studies suggest is the case for stickleback populations living in other discrete habitats where exposure differences are very pronounced. Of course, the evidence presented here is only correlative, and definitive evidence for local adaptation needs to come from experimental infections and reciprocal transplants. There are a number of studies indicating that stickleback are locally adapted to their parasites (Kalbe and Kurtz 2006; De Roij et al. 2010), consistent with our interpretation of our data. Additionally, a reciprocal transplant experiment between one of the lakes studied here (Roberts) and its adjoining stream revealed higher parasite loads of fish transplanted to nonnative than to their native habitat (W. E. Stutz, unpublished manuscript). However, our models suggest a more specific prediction that has not yet been tested in field experiments: fish from populations with low prevalence of a given parasite (cestode or nematode) should be more resistant to that parasite than populations in which the parasite is more prevalent. Altogether, our study points the way towards future work integrating the ecological factors that determine exposure and infection within and among populations.

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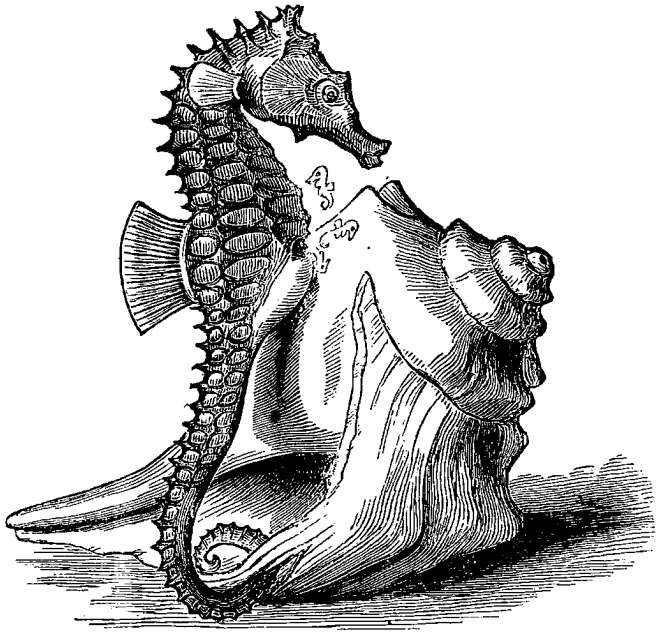

"The male [Sea-horse], even though pressed by hunger, will not molest his offspring—a remarkable fact, when we reflect that generally fishes have no scruples against devouring any fry, even their own. This trait of the male Sea-horse is found in the male Stickleback. The former is not very demonstrative [...]; but the latter is highly so, even to vindictiveness, as I have seen him severely punish the female in his anxiety for the safety of the spawn." From "The Sea-Horse and its Young" by Samuel Lockwood (*The American Naturalist*, 1867, 1: 225–234).